REMARKS

Amendments to the Claims

Claims 1, 2, 4, 5 and 7-17 were pending.

Claims 13-15 has been withdrawn.

Claims 2, 4 and 10 have been canceled without prejudice.

Claims 1, 5, 11, 12, 16 and 17 have been amended.

Claims 1, 11 and 12 have been amended to delete the phrase "the Fisher ratio is determined without the use of a prior probability."

Claims 1, 11 and 12 have been further amended to recite that the genes and/or proteins are selected "by a supervised learning method." Support for this amendment can be found in the Specification, for example, at page 6, lines 17 through 29.

Claim 1 has been amended to incorporate the elements in Claims 2 and 4. Specifically, Claim 1 has been amended to recite "liver" and to reflect the "five" stages of hepatocellular carcinoma (HCC), namely "non-cancerous liver (L0), pre-cancerous liver (L1), well differentiated hepatocellular carcinoma (HCC) (G1), moderately differentiated HCC (G2) and poorly differentiated HCC (G3). Support for this amendment can be found in the Specification, for example, at page 5, line 31 through page 6, line 15 and the original Claim 2 as filed.

Claim 1 has been further amended to recite: "the genes and/or proteins that are differentially expressed between (1) L0 and L1, (2) L1 and G1, (3) G1 and G2, and (4) G2 andG3." Support for this amendment can be found in the Specification, for example, at page 23, line 20 through page 30, line 10; and the original Claim 4 as filed.

Claim 5 has been amended to depend from Claim 1.

Claim 16 has been amended to delete steps (ii) and (iii).

Claim 17 has been amended to delete step (ii).

Claims 18 and 19 have been added. Support for these claims can be found in the Specification, for example, page 6, lines 24-29; page 10, line 27 through page 11, line 8; and page 33, lines 2-8.

No new matter has been added. Entry of these amendments is respectfully requested.

Disposition of Claim 5

Although Claim 5 was examined and indicated as pending in the Office Action, Claim 5 was omitted from the list of the pending claims in the Office Action Summary. Applicants respectfully request the status of Claim 5 be corrected in the Office Action Summary.

Rejection of Claims 1, 2, 4, 5, 7-12, 16 and 17 Under 35 U.S.C. § 112, First Paragraph

Claims 1, 2, 4, 5, 7-12, 16 and 17 have been rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement.

Although Applicants disagree, in the interest of furthering prosecution, independent Claims 1, 11 and 12 have been amended to delete the phrase "the Fisher ratio is determined without the use of a prior probability," rendering the rejection moot against these claims and other pending claims that depend from these claims. Further, Claims 2, 4 and 10 have been canceled without prejudice, rendering the rejection of these claims moot.

Rejection of Claims 1, 2, 4, 5 and 7-9 Under 35 U.S.C. § 103(a)

Claims 1, 2, 4, 5 and 7-9 stand rejected as being unpatentable under 35 U.S.C. § 103(a) over Okabe *et al.* (Cancer Research, 61:2129-2137 (2001); hereinafter, "Okabe") in view of Adorjan *et al.* (U.S. Patent Publication 2002/0192686 A1; hereinafter, "Adorjan"). The Office Action stated that it would have been obvious to a person having ordinary skill in the art to combine the features disclosed in the cited references to arrive at the present invention.

While Applicants maintain the arguments in Applicants' previous response submitted on June 3, 2010, in the interest of furthering prosecution, Applicants have amended the claims as noted above.

Applicants assert that the pending claims, as amended, are not rendered obvious over Okabe in view of Adorjan for at least the reasons set forth below.

Teachings of the Cited References

As noted in the Office Action at page 4, first paragraph, Okabe teaches the importance of analyzing microarray genome-wide expression profiles in human hepatocellular carcinoma (HCC) using a "cluster analysis." By employing the Mann-Whitney statistical method, Okabe identified a set of genes highly associated with the HCC progression between "well-

differentiated tumors" and "moderately-to-poorly differentiated tumors" (*see* Okabe, pages 2136-2137).

Adorjan teaches a method for identifying an epigenetic feature (*i.e.*, DNA methylation between two particular types of cancer: acute myeloid leukemia (AML); and acute lymphobalastic leukemia (ALL)). To identify DNA methylation profiles associated with AML and ALL from a large scale genome-wide methylation data, Adorjan implemented various types of statistical selection criteria, including the Fisher criterion (*see*, Adorjan, paragraphs [104] and [105]).

Subject Matter of the Present Invention

The present invention is directed to *in vitro* methods of defining the differentiation grade of a tumor with genes and/or proteins representing the differences between two consecutive sequential groups of HCC, progressing from non-cancer to late stage cancer. Differentiation states are divided into 5 grades (*see* the Specification at page 5, line 31; and Claim 1). Namely, the groups between which the gene expression is compared include: (1) non-cancerous liver (L0) and pre-cancerous liver (L1); (2) pre-cancerous liver (L1) and well differentiated hepatocellular carcinoma (HCC) (G1); (3) well differentiated HCC (G1) and moderately differentiated HCC (G2); and (4) moderately differentiated HCC (G2) and poorly differentiated HCC (G3). Notably, the genes differentially expressed between two groups are selected in descending order of the Fisher ratio, based on a supervised learning.

Applicants submit herewith Exhibit A that depicts the differences between the supervised learning and the unsupervised learning. In the supervised learning, one determines the type of training examples and obtains a training set that is representative of the real function, namely, a set of input objects and corresponding outputs. Then, one determines how to represent the input objects and the structure of the learned function and the learning algorithm is determined accordingly. One, then, finalizes the design and runs the learning algorithm on the gathered training set with or without a specific parameter which can be further adjusted by optimizing performance on a validation set of the training set, or via cross-validation. One then, evaluates the accuracy of the learned function. The performance of the resulting function is measured on a test set that is separate from the training set.

In the present case, the validity of the selected genes and of the differentiation grade of test samples were also confirmed by unsupervised cluster approaches, namely the minimum distance classifier (*see* Example 13; and Fig. 2) and the self-organizing map (SOM) (*see* Example 14; and Fig. 3).

For example, Applicants created a minimum distance classifier with the selected genes for each individual transition between all five sequential stages of HCC (*i.e.*, L0, L1, G1, G2 and G3). The resulting classifier classified the test samples with the accuracy of 92% (Fig. 2a; L0 v. L1), 98% (Fig. 2b; L1 v. G1), 84% (Fig. 2c; G1 v. G2), and 100% (Fig. 2d; G2 v. G3) (the Specification at page 31, lines 7-9).

With respect to the validation by the SOM, another unsupervised learning approach, when test sample were visualized using all the genes in the SOM, the test samples were classified as each of L0, L1, G1, G2 and G3 converge and form clusters (*see* Fig. 3 of the present Application).

These results vividly demonstrate that the selected genes identified by the supervised learning in descending order of the Fisher ratio are indeed the genes that define the differentiation grade. The results also conclusively validate that the method described in the application can be used to diagnose the differential grade of HCC of an unknown liver sample with a sufficient discrimination rate.

Non-obviousness of the Present Invention

The present invention is not obvious because the combined teachings of the cited references do not teach or suggest all elements of the present invention. The present invention is directed to identifying and selecting genes and/or proteins differentially expressed in each transition between the five sequential stages of HCC, particularly based on the supervised learning and the Fisher ratio. In contrast, the teachings of Okabe are based on the unsupervised learning method, namely a cluster analysis and do not provide any inference of success on the use of the supervised learning method to select genes and/or proteins based on their expression patterns. Nor does it teach the identification genes differentially expressed in each transition between the five sequential stages of HCC. The teachings of Adorjan does not compensate for the deficiencies in Okabe because Adorjan merely teaches the Fisher criterion. Thus, there are

essential differences between the present invention and the combine teachings of the cited references as elaborated below.

In the supervised learning method, the accuracy of the results can be easily verified or cross-validated because the information regarding into which grade (any of L0, L1, G1, G2, and G3) each case should be classified is already available to the user. In contrast, the unsupervised learning as used in Okabe's cluster analysis is carried out without any prior information about the case, and thus, quantitative assessment such as an estimate of discrimination rate is not available for further analysis.

Although the present application describes the use of the minimum distance classifier and the SOM, which utilize the unsupervised learning cluster analysis, these were only implemented to validate of the genes that were already identified and selected by the supervised learning method in the descending order of the Fisher ratio.

Finally, the Fisher ratio described in Adorjan is not applicable to the present invention because the formula for determining the Fisher ratio described in Adorjan is statistically inappropriate (*see* Adorjan at page 9, lines 3-7; paragraph [0104]). The formula described by Adorjan is as follows:

$$J(k)=\frac{(m_k^{\ A}-m_k^{\ B})}{(s_k^{\ 2A}+s_k^{\ 2B})}$$

Specifically, the denominator of the above formula is the sum of variance, and thus, is always positive. On the other hand, the numerator is the difference between the mean of sample A and the mean of sample B and can be either a positive or negative value. Therefore, the value of the formula, *J(k)*, itself can be either positive or negative.

In contrast, the Fisher ratio of the present invention is determined by the formula set forth below:

$$F(j)=\frac{(\hat{\mu}_j(A)-\hat{\mu}_j(B))^2}{\hat{\sigma}_j^2(A)+\hat{\sigma}_j^2(B)}$$

(*see* also the Specification page 9, line 29)

The numerator of the formula described by Applicants is the square of the differences between the mean of sample A and the mean of sample B, which always results in a positive value.

Because the Fisher ratio serves as an evaluation criterion, the value must not be negative. If one of ordinary skill in the art uses the formula provided in Adorjan for determining the ratio, the evaluation would be greatly different from that of the present invention.

Thus, the combined teachings of the cited references do not teach or suggest the present invention as claimed. Further, because there is no teaching or suggestion, there is no motivation to combine the teachings of Okabe and Adorjan to arrive at the present invention with a reasonable expectation of success. Accordingly, a *prima facie* case of obviousness has not been established over Okabe and Adorjan. Reconsideration and withdrawal of the rejection are respectfully requested.

Rejection of Claims 1, 2, 4, 5 and 7-9 Under 35 U.S.C. § 103(a)

Claims 1, 2, 4, 5 and 7-9 stand rejected as being unpatentable under 35 U.S.C. § 103(a) over Okabe in view of Adorjan and Bloch (U.S. Patent No. 6,728,642 B2; hereinafter, "Bloch").

The deficiencies in the combined teachings of Okabe and Adorjan are discussed in detail above.

Bloch was cited in the Office Action for its teachings relating to the minimum distance classifier as a method of cluster identification based on an unsupervised learning (*see* Bloch, col. 9, lines 23-25).

Bloch does not compensate for the deficiencies in Okabe and Adorjan. Bloch does not teach or suggest the use of the supervised learning method to identify and select genes and/or proteins differentially expressed between two groups. Nor does Bloch teach or suggest the use of a formula for determining the Fisher ratio that always results in a positive value.

Further, Bloch teaches that the minimum distance classifier as an example to explain the cluster identification. Bloch uses the minimum distance classifier to identify clusters and to elucidate the relationships between the clustered genes, while Applicants of the present application used the minimum distance classifier to validate the genes selected by the Fisher ratio based on the supervised learning. Thus, one of ordinary skill in the art would not have been motivated to implement the minimum distance classifier described in Bloch to arrive at the present invention.

Finally, it is stated in the Office Action that: "[Bloch et al.] teaches organizing clusters for presentation by illustrating classified genes into self-organizing maps (see Figures 10-11, in particular)..." However, Figures 10 and 11 presented in Bloch are not the results of the SOM. Rather, they are the results of the hierarchical clustering which is an entirely different analysis from the SOM.

Therefore, a *prima facie* case of obviousness has not been established by the combined teachings of Okabe, Adorjan and Bloch. Reconsideration and withdrawal of the rejection are respectfully requested.

Rejection of Claims 1, 2, 4, 5 and 7-11 Under 35 U.S.C. § 103(a)

Claims 1, 2, 4, 5 and 7-11 have been rejected as being unpatentable under 35 U.S.C. § 103(a) over Okabe in view of Adorjan and Tang (*World J. Gastroenterol.*, 7:445-454 (2001); hereinafter, "Tang").

The deficiencies in the combined teachings of Okabe and Adorjan are discussed in detail above.

Tang reference is a review article that teaches the causation of HCC in general. Tang teaches that hepatitis C virus (HCV) infection is a major factor for HCC and makes a general statement that: "Tremendous works have been done at the molecular level, which will provide clues for biomarker of HCC progression as well as targets for intervention." (Tang, Abstract).

Unlike the statement in the Office Action[1], Tang does not motivate one of ordinary skill in the art to arrive at the present invention because Tang merely makes a rather general statement that the works done at the time would provide clues for biomarkers of HCC progression. As noted above, the present invention is a comprehensive analysis on gene expression specifically directed to the differentiation grade of HCV-HCC encompassing all five different stages of HCC. The present invention identifies specific genes and/or proteins that define the differentiation grade of HCC in descending order of the Fisher ratio, based on the supervised learning. The present invention also provides diagnostic methods for the successful discrimination of cases using the selected genes. Tang does not teach or suggest the use of supervised learning,

[1] "Tang further teaches motivation to identify biomarkers for HCC progression (*see* Abstract, in particular)." (Office Action at page 12, 4th paragraph).

particularly in identifying and selecting genes that are differentially expressed in each transition between the five sequential stages of HCC. Nor does it teach or suggest the use of a formula for determining the Fisher ratio that always results in a positive value. Simply, Tang does not compensate for the deficiencies in the combined teachings of Okabe and Adorjan. As such, a *prima facie* case of obviousness has not been established over the teachings of Okabe, Adorjan and Tang. Reconsideration and withdrawal of the rejection are respectfully requested.

Rejection of Claims 1, 2, 4, 5, 7-12, 16 and 17 Under 35 U.S.C. § 103

Claims 1, 2, 4, 5, 7-12, 16 and 17 have been rejected as being unpatentable under 35 U.S.C. § 103(a) over Okabe in view of Adorjan, Tang and Bloch.

The deficiencies of the combined teachings of Okabe, Adorjan and Tang are discussed above.

The deficiencies of the teachings in Bloch are also discussed above.

The combined teachings of Okabe, Adorjan, Tang and Bloch does not teach or suggest the use of the supervised learning method in identifying and selecting genes and/or proteins that are differentially expressed in each transition between the five sequential stages of HCC. Nor does it teach or suggest the use of a formula for determining the Fisher ratio that always results in a positive value.

Because the combined teachings of Okabe, Adorjan, Tang and Bloch does not teach or suggest the present invention, a *prima facie* case of obviousness has not been established. Reconsideration and withdrawal of the rejection are respectfully requested.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned.

Respectfully submitted,

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